Endometrial biopsy-induced gene modulation: first evidence for the expression of bladder-transmembranal uroplakin Ib in human endometrium

Yael Kalma, Ph.D.,[a] *Irit Granot, Ph.D.,*[b] *Yulia Gnainsky, Ph.D.,*[a] *Yuval Or, M.D.,*[b] *Bernard Czernobilsky, M.D.,*[c] *Nava Dekel, Ph.D.,*[a] *and Amihai Barash, M.D.*[b]

[a] Department of Biological Regulation, Weizmann Institute of Science, Rehovot; [b] IVF Unit, Department of Obstetrics and Gynecology, Kaplan Medical Center (Affiliated with the Medical School of the Hebrew University and Hadassah, Jerusalem), Rehovot; and [c] Patho-Lab Diagnostics, Ness-Ziona, Israel

Objective: To explore the possibility that endometrial injury modulates the expression of specific genes that may increase uterine receptivity.
Design: Controlled clinical study.
Setting: Clinical IVF unit and academic research center.
Patient(s): IVF patients with 28- to 30-day menstrual cycles.
Intervention(s): Endometrial biopsies from two groups of patients were collected on days 20–21 of their spontaneous menstrual cycle. The experimental, but not the control, group underwent biopsies on days 11–13 and 21–24 of their preceding cycle.
Main Outcome Measure(s): Global endometrial gene expression and specific analysis of uroplakin Ib (UPIb) mRNA level throughout the menstrual cycle.
Result(s): Local injury modulated the expression of a wide variety of genes. One of the prominently up-regulated genes was the bladder transmembranal protein, UPIb, whose expression by the endometrium is shown here for the first time. Endometrial UPIb mRNA increases after biopsy in the same cycle wct 2with an additional elevation in the following cycle. Immunohistochemical analysis localized the UPIb protein to the glandular-epithelial cells. Genes encoding other membrane proteins such as adipose differentiation-related protein and mucin 1, transmembrane, were also up-regulated.
Conclusion(s): The biopsy-induced increase in the expression of UPIb and other genes encoding membrane proteins supports the possible importance of the membrane structure and stability during implantation. The specific role of UPIb in uterine receptivity should be elucidated. (Fertil Steril® 2009;91:1042–9.e9. ©2009 by American Society for Reproductive Medicine.)

Key Words: Biopsy, endometrium, implantation, in vitro fertilization, UPIb

The formation of a receptive endometrium involves morphological and functional changes that are induced by the sex steroids estrogen and progesterone (P). Estrogen is secreted mainly during the first half of the menstrual cycle, which is referred to as the proliferative phase. P is the predominant sex steroid in the following secretory phase of the cycle, which is characterized by the formation of large glandules that secrete high amounts of cytokines and growth factors (GFs). In humans, the uterus becomes receptive during the midsecretory phase of the menstrual cycle (days 19–23), which is commonly known as the implantation window and which spans 7–10 days after the LH surge (1).

Received August 26, 2007; revised and accepted January 11, 2008; published online March 19, 2008.
Yael Kalma and Irit Granot contributed equally to this paper.
This work was supported by the Dwek Fund for Biomedical Research.
All authors have nothing to disclose.
Reprint requests: Irit Granot, Ph.D., IVF Unit, Department of Obstetrics and Gynecology, Kaplan Medical Center, Rehovot, 76100, Israel (FAX: 972-8-9441-217; E-mail: irit_g@clalit.org.il).

The morphological changes during the implantation window include transformation of the fibroblast-like endometrial stromal cells into larger and rounded decidual cells (decidualization) (2) and emergence of large apical protrusions (pinopodes) and microvilli on the luminal epithelium (3). In parallel, modulations in the expression of different cytokines, GFs, transcription factors, and prostaglandins (1, 3) take place. Specifically, an increase in interleukin-11 (IL-11) and leukemia inhibitory factor (LIF) expression was observed in human endometrial cells during the midsecretory phase (4, 5). Along this line, in vitro studies suggested that PGE2 and relaxin act via IL-11 to phosphorylate Stat3, which enhances P-induced decidualization in cultured human stromal cells (6). A decrease in genes from the HOX cluster such as hoxc10, hoxc11, hoxd10, and hoxd11 during the implantation window, which was also observed, suggests that these transcriptional repressors may interfere with the preparation of the endometrium for implantation (7).

Global microarray analysis, which was employed in the search for implantation markers, revealed a large number of

0015-0282/09/$36.00
doi:10.1016/j.fertnstert.2008.01.043

genes that were expressed differentially in human endometrium during the implantation window (8–13). These and other studies provide correlative evidence for the possible involvement of some indicated genes in implantation; however, their specific function is yet to be determined. To date, functional evidence has been reported only in the mouse experimental model. This limited information was generated in knockout experiments demonstrating that endometrial expression of LIF (14), hoxa 10, and hoxa 11 (15, 16) is obligatory for endometrial preparation for embryo implantation. In addition, blocking uterine production of proprotein convertase 6 in mice, using morpholino antisense, also resulted in inhibition of implantation (17).

There is no evidence connecting specific endometrial genes to impaired fertility in human. For example, studies that compared the expression of IL-11 and LIF in the endometrium of fertile and infertile women with endometriosis failed to generate consistent results. Dimitriadis et al. (18) demonstrated a reduction in IL-11 and LIF expression in women with endometriosis. However, Mikolajczyk et al. (19) could not demonstrate a difference in IL-11 and LIF mRNA levels between fertile and infertile women.

Although many fertility disorders have been overcome by a variety of assisted reproductive technologies, implantation remains the rate-limiting step for the success of IVF. Therefore, further studies are needed to provide clear evidence of specific genes that are involved in decidualization and implantation. In our previous study, we demonstrated that local injury of the endometrium in IVF patients substantially increases its receptivity. Specifically, endometrial biopsies taken during the spontaneous cycle that preceded the IVF treatment more than doubled the rates of implantation, clinical pregnancies, and live births (20). Such a favorable influence of local injury on the endometrium was later confirmed by Raziel et al. (21), who showed a significant increase in the success of IVF treatments in a population of patients with a high order of previous implantation failures. A more recent study further demonstrated that local injury of the endometrium executed in IVF patients during their cycle of treatment, before ovum retrieval, gains the same effect of increasing implantation and clinical pregnancy rates (22).

In an attempt to identify the genes that are involved in the local injury–induced endometrial receptivity, we compared the endometrium of biopsy-treated and -untreated patients using microarray technology. Our results revealed that this local injury modulated a variety of genes that belong to different biologically functional groups. The most prominent up-regulation was demonstrated by uroplakin Ib (UPIb), which is a member of a family of four glycoproteins that strengthen and stabilize the apical surface of the mammalian bladder (23, 24). We provide the first evidence that UPIb is expressed by human endometrium. We further show that the expression of UPIb is up-regulated after biopsy treatment. This increase in UPIb is sustained and even elevated in the following menstrual cycle.

MATERIALS AND METHODS

Sample Collection

Endometrial samples were collected on days 20–21 of the spontaneous menstrual cycle, which represents the receptive window of the uterus. These samples were taken from two groups of IVF patients with 28- to 30-day menstrual cycles. The experimental group included patients who underwent biopsies on days 11–13 and 20–24 of their previous spontaneous cycle, according to the protocol practiced in our previous studies (20, 25). No such treatment was performed in the control group of patients. All patients were subjected to the routine IVF protocol of treatment at the following cycle. The average age and the number of previous failing IVF cycles of the two groups were not significantly different. From the experimental group, we selected samples of four biopsy-treated patients who conceived in the subsequent IVF treatment. From the control group, samples of four untreated patients who failed to conceive were selected for analysis.

Part of each sample was examined pathologically to confirm correspondence to the relevant phase of the menstrual cycle.

The protocol of this study was approved by the Kaplan Medical Center Review Board on the use of Human Subjects in Medical Research in accordance with the Helsinki Declaration and by the Israeli Ministry of Health in Jerusalem.

IVF Protocol of Treatment

Hormone stimulation, oocyte retrieval and insemination, and embryo culture and transfer were performed as described elsewhere, with minor modifications (20). Briefly, patients were treated with GnRH analog (GnRHa) buserelin scetate (Superfact, Hoechst AG, Frankfurt, Germany) or triptorelin acetate (Decapeptyl, Ferring GmbH, Kiel, Germany) for pituitary down-regulation and endogenous gonadotropin depletion. For stimulation of follicular growth, patients were treated with Menogon (Ferring, GmbH, Kiel, Germany) or with a combination of Menogon and Gonal F (Serono, Basel, Switzerland). Oocyte maturation was induced by Ovitrelle (Choriogonadotropin alpha, Serono, Basel, Switzerland), which was administered upon ultrasound detection of at least two 18-mm lead follicles. Oocytes were retrieved 32–36 hours after Ovitrelle administration and exposed to spermatozoa for insemination. Oocytes exhibiting two pronuclei at 16–20 hours after insemination were further incubated for embryonic development. Transfer of 4- to 8-cell embryos was performed 2–3 days after oocyte retrieval, respectively.

Gene Expression Profiling

Four endometrial samples of each of the two groups mentioned above were compared by profiling their global gene expression using microarray analysis. For microarray analysis, total RNA from each individual sample was extracted using the Tri Reagent method (Molecular Research

TABLE 1

Primers for PCR.

Gene	Sequence
Kiaa0367	5′-CGCCTGACTTGTGGATAGATGC-3′
	5′-CTGTCGGAAGCCTGAGAATTGC-3′
BCL2/adenovirus E1B 19kDa interacting protein 3-like (BNIP3L)	5′-ACCATCCTCATCCTCCATCC -3′
	5′-GGAATGTTTTCGGGTCTACTGG -3′
Dual oxidase 1 (DUOX1)	5′-GTGACAGATGTGCCAGATACCC-3′
	5′-GCTGACGGATGACTTGAAGACC-3′
Annexin A3 (ANXA3)	5′-GACAAGCAGGCAAATGAAGG -3′
	5′-TTTGTCTTCATCCGTGCCCC -3′
Crystallin, alpha B (CRYAB)	5′-CGCCTCTTTGACCAGTTCTTCG -3′
	5′-TGCTTCACATCCAGGTTGACAG -3′
Galanin (Gal)	5′-GCTCGCCTCCCTCCTCCTC-3′
	5′-CTTGTCGCTGAATGACCTGTGG -3′
Cystatin SN (CST1)	5′-TTCTGGCTGTTCATGGAAGG -3′
	5′-AATGATGAGTGGGTACAGCG -3′
ADAM metallopeptidase with thrombospondin type 1 motif, 8 (ADAMTS8)	5′-TGCCAAGCCCTGCGAAAGC-3′
	5′-GCCCCATACCCTCTCCTCTTCC-3′
Cysteine-rich secretory protein 3 (CRISP3)	5′-AGTGCTGTTGTTCCTGGTTGC -3′
	5′-TGCTCTCCTCAGTTCATTGTGC-3′
Tissue factor pathway inhibitor 2 (TFPI2)	5′-TGTGGACGACCAGTGTGAGG-3′
	5′-CGCAGAAGCCCATACAAGTAGC-3′
UPIb	5′-TGCCTCTTCTGCCTGTCTGTTC-3′
	5′-GTCTCGTTGTGTTGCTGCTGTG -3′
Toll-like receptor 5 (TLR5)	5′-TCTGTTCCTCATGACCATCC -3′
	5′-GAAGAAACCAGCCAACATCC -3′
ADFP	5′-ACTGGCTGGTAGGTCCCTTT-3′
	5′-TGCTTCCCAATTTAGGGTTG -3′
LAMP2B	5′-GGTTAATGGCTCCGTTTTCA -3′
	5′-TCATCCAGCGAACACTCTTG -3′
Mucin 1, cell surface associated (MUC1)	5′-AGACGTCAGCGTGAGTGATG -3′
	5′-CAGCTGCCCGTAGTTCTTTC -3′
Ribosomal protein S16 (S16)	5′-GCGGCAATGGTCTCATCAAGG -3′
	5′-CGGATGTCTACACCAGCAAATCG -3′

Note: Sequences of primers were used for validation of array results. To verify microarray results, PCR was performed using first-strand cDNA. Primers for selected-up regulated genes were designed using Beacon Designer software (Premier Biosoft International, Palo Alto, CA).

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

Center, Inc., Cincinnati, OH). Double-strand cDNA was synthesized from each RNA sample by reverse transcription. Biotin-labeled cRNA was produced from the cDNA and was probed separately to the U133A Affymetrix GeneChip containing probes of 20,000 human genes. Evaluation of the data was performed using the GeneSpring analysis software (Agilent Technologies, Inc., Santa Clara, CA).

Reverse Transcription–Polymerase Chain Reaction (RT-PCR)

Verification of the expression of representative genes and further analysis of selected genes was performed by RT-PCR analysis. RT-PCR was performed on total RNA prepared by the Tri-Reagent method. Samples of 7.5 μg of RNA were reverse transcribed to cDNA using M-MLV reverse transcriptase (Promega, Madison, WI) and oligo dT (Pharmacia, London, UK). PCR was performed on 1 μL of the 20-μL cDNA sample. Specific primer sets (Table 1) were synthesized and were used at 10 pmol per reaction.

Immunohistochemistry

Immunohistochemical labeling of paraffin-embedded endometrium sections was carried out using an indirect-streptavidin ABC immunoperoxidase technique. After

deparaffinization, unstained slides were microwave heated in antigen retrieval solution (10 mM sodium citrate) for 10 minutes. Anti-UPIb antibody (Santa-Cruz sc15174) was applied at a 1:40 dilution. Anti-goat IgG secondary antibody and Vectastain ABC kit was purchased from Vector Laboratories (Burlingame, CA). Diaminobenzidine was used as a substrate.

RESULTS

Analysis of Array Results

To identify genes whose expression is modulated as a result of local injury of the endometrium, total RNA was extracted from endometrial samples of four control patients and four biopsy-treated patients who conceived at the subsequent IVF treatment. Each RNA extract was separately hybridized to a chip. The fold change values were calculated as the ratio between the mean values of the four experimental and control samples.

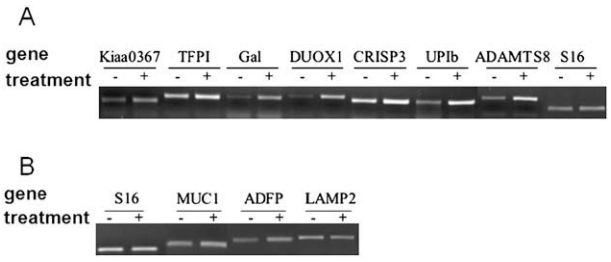
Statistical analysis of the array results revealed a 2-to 10-fold increase in the expression of 183 genes in the endometrial samples of the biopsy-treated patients. The expression of 39 genes in these samples was down-regulated by at least twofold. Cluster analysis based on DAVID Bioinformatics Resources (http://david.abcc.ncifcrf.gov) was performed, allowing the classification of the increased or decreased genes into different functional groups (see Appendices 1 and 2, available in the online version). Among the up-regulated genes, the largest functional groups included those that encode for proteins involved in cellular lipid metabolism (n = 19), cell communication/signal transduction (n = 17), transport (n = 23), cell adhesion (n = 15), and cell organization/cytoskeleton proteins (n = 12) and genes related to transcription regulation/DNA metabolism (n = 21). Some other smaller groups include genes that encode for proteins related to the immune/wound healing response (n = 5), cell cycle (n = 5), extracellular matrix/proteolysis (n = 6), and cell surface/membrane proteins (n = 4). Classification of the down-regulated genes revealed functional groups such as cell cycle proteins (n = 9), communication/signal transduction molecules (n = 7), and extracellular matrix/proteolysis molecules (n = 4). In several cases, different genes from the same functional group were detected in both the up- and down-regulated genes.

Fifteen up-regulated genes were selected for confirmation of the microarray results. For this purpose, genes exhibiting the highest increase and/or those possibly relevant to implantation were selected. RT-PCR analysis of an RNA pool showed an increase in mRNA levels of 13 out of the 15 selected genes. The primer sets used for the RT-PCR are shown in Table 1. Representative results of seven genes are shown in Figure 1A. The highest up-regulation was demonstrated for UPIb.

The UPIb gene was present twice on the chip and showed up-regulation in samples of biopsy-treated patients in both cases by 7.4- and 6.7-fold. Other genes from the membrane protein group such as adipose differentiation-related protein (ADFP), mucin 1, transmembrane (MUC1), and lysosomal-associated membrane protein 2 (LAMP2) were also up-regulated (Table 2). Validation of the modulation of these genes by RT-PCR (Fig. 1B) confirmed the increase in mRNA levels of ADFP and MUC1 in samples of biopsy-treated patients and failed to demonstrate alternation in the expression of LAMP2.

FIGURE 1

Validation of selected genes by RT-PCR. cDNAs from endometrial samples of biopsy-treated (+) and control (−) patients were prepared and grouped into two pools. PCR was conducted with specific primer sets for the selected genes: dual oxidase 1 (DUOX1), cysteine-rich secretory protein 3 (CRISP3), Kiaa0367, tissue factor pathway inhibitor 2 (TFPI2), UPIb, Galanin (Gal), ADAM metallopeptidase with thrombospondin type 1 motif and 8 (ADAMTS8) are shown in panel **A**. ADFP, MUC1, and LAMP2, which encode membrane proteins, are shown in panel **B**. S-16 served as an internal standard.



Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

Spatiotemporal Pattern of UPIb Expression

The expression of UPIb was further examined on days 12–13 of the menstrual cycle, which represent the late proliferative phase, and on days 21–24 of the secretory phase, which represent the implantation window. An increase in UPIb mRNA levels on days 21–24 of the cycle was observed in 12 out of the 15 patients examined. Representative results of six patients are shown in Figure 2A. Interestingly, other genes that were up-regulated in the array analysis, such as crystallin alpha B and apolipoprotein D (APOD), were previously shown to exhibit a similar pattern of expression throughout the menstrual cycle (10, 13, 26).

To further assess the effect of the biopsy treatment on UPIb expression, we collected endometrial samples from 11 patients, three of whom were included in the biopsy-treated group analyzed by microarray. Three endometrial samples were taken from each patient, on days 11–14 and 21–24 of the first cycle and on days 20–21 of the following cycle, which represents the cycle of IVF treatment. This analysis revealed that in addition to the increase during the same cycle, a further increase in UPIb expression was observed on days 20–21 of the following cycle. Such an increase was observed

TABLE 2

Genes up-regulated by endometrial biopsy.

No.	Description	Families	Accession no.	Fold increase	*P*
1	KIAA0367	Antiapoptosis	NM_015225	5.7/3.2	<.01
2	BCL2/adenovirus E1B 19kDa interacting protein 3-like (BNIP3L)	Antiapoptosis	NM_004331	2.5	<.01
3	Dual oxidase 1 (DUOX1)	Cell communication/ signal transduction	NM_017434 NM_175940	10.3	<.01
4	Annexin A3 (ANXA3)	Cell communication/ signal transduction	NM_005139	2.3	<.01
5	Crystallin, alpha B (CRYAB)	Cell communication/ signal transduction	NM_001885	3.1	<.01
6	Galanin (Gal)	Cell communication/ signal transduction	NM_015973	5.2	<.01
7	Cystatin SN (CST1)	Cell communication/ signal transduction	NM_001898	3.4	<.01
8	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 8 (ADAMTS8)	Extracellular matrix/ proteolysis	NM_007037	2.6/6.9	<.01
9	Cysteine-rich secretory protein 3 (CRISP3)	Cell adhesion molecules	NM_006061	8.7	.0178
10	Tissue factor pathway inhibitor 2 (TFPI2)	Immune/wound response	NM_006528	5.0	.0164
11	Toll-like receptor 5 (TLR5)	Immune/wound response	NM_003268	2.5	<.01
12	Uroplakin 1B (UPK1B)	Cell surface/ membrane	NM_006952	7.4/6.7	<.01
13	ADFP	Cell surface/ membrane	NM_001122	2.3	<.01
14	LAMP2	Cell surface/ membrane	NM_002294 NM_013995	2.1	<.01
15	MUC1	Cell surface/ membrane	NM_001018016 NM_001018017 NM_001018021 NM_002456	2.1	.029

Note: Genes were up-regulated by endometrial biopsy and confirmed by RT-PCR. Total RNA was extracted from endometrial samples of four biopsy-treated patients who conceived in the subsequent IVF treatment and four control patients. Each RNA extract was separately hybridized to a chip. The fold change values were calculated as the ratio between the mean values of the four treated and control samples. Representative genes, of which the fold of increase was confirmed by RT-PCR, are presented. In cases that genes were present twice on the chip, both values are presented. The complete table containing all the genes that were up-regulated in endometrial biopsy treated–patients is included in the appendices.

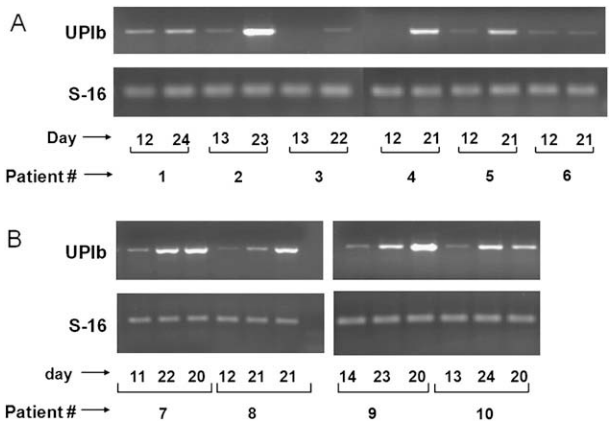
Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

in six out of the 11 patients. Representative results of four patients are shown in Figure 2B.

Immunohistochemical analysis of the endometrium (Fig. 3A) shows that UPIb is localized on the apical region of the cytoplasmic membranes of the glandular epithelial cells as well as on the membranes of their secretory vacuoles. In agreement with the PCR results, an increase in UPIb protein was observed from the proliferative to the secretory phase of the cycle (Fig. 3B).

FIGURE 2

The effect of local injury of the endometrium on the expression of UPIb. (**A**) Two endometrial biopsies were taken from each patient, one at the late-proliferative (days 12–13) and the other at the midsecretory (days 21–24) phases and were subjected to RT-PCR with specific primers to UPIb. S-16 served as an internal standard. Representative results of six patients are presented. (**B**) Three endometrial biopsies were taken from each patient: two at different phases of the same cycle (late-proliferative, days 11–14; and midsecretory, days 21–24) and an additional one at the following cycle (days 20–21). These samples were subjected to RT-PCR with specific primers to UPIb. S-16 served as an internal standard. Representative results of four patients are presented.



Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

DISCUSSION

Biopsy treatment substantially improved the success rate of implantation in IVF patients (20–22). We demonstrate an increase of 2- to 10-fold in the expression of 183 genes that was observed in the patients who underwent endometrial biopsy and conceived in the subsequent IVF treatment. The expression of 39 genes in these women was down-regulated. One of the most prominently up-regulated genes is UPIb. Further characterization revealed an elevation of the UPIb mRNA levels at the implantation window (days 21–24) of the spontaneous menstrual cycle, with an additional increase observed on days 20–21 of the cycle, which follow the biopsy treatment. Immunohistochemical analysis localized UPIb to the endometrial gland epithelium and showed an increase from the proliferative to the secretory phase of the cycle that corresponds to the expression of its mRNA.

Among the other genes that were up-regulated in the endometrial samples of the biopsy-treated patients, MUC1, crystallin alpha B, APOD, and PLA_2 are included. Changes in these genes during the transition from the proliferative to the secretory phase of the menstrual cycle has been demonstrated by previous studies that used DNA microarray analysis (26, 27). Furthermore, experiments on mice have shown that females lacking PLA_2 exhibit deferred implantation and reduction in litter size, which is caused by the absence of prostaglandins (27). These results support our hypothesis that local injury increases endometrial receptivity by modulation of the expression of a variety of genes that are involved in the preparation of the endometrium for embryo implantation. It may be possible that IVF patients who fail to conceive even though high-quality embryos are transferred into their uterus are unable to elevate the expression of genes related to endometrial receptivity in a spontaneous manner. In these cases, local injury of the endometrium introduced by the biopsy may facilitate the endometrial response.

In this study, we chose to focus on UPIb, the expression of which showed one of the most prominent elevations in the endometrial samples of the biopsy-treated patients. UPIb was present twice on the chip and in both cases was significantly up-regulated. This gene has not been previously shown to be related to the receptivity of the endometrium. In fact, the expression of UPIb, in the endometrium is reported here for the first time.

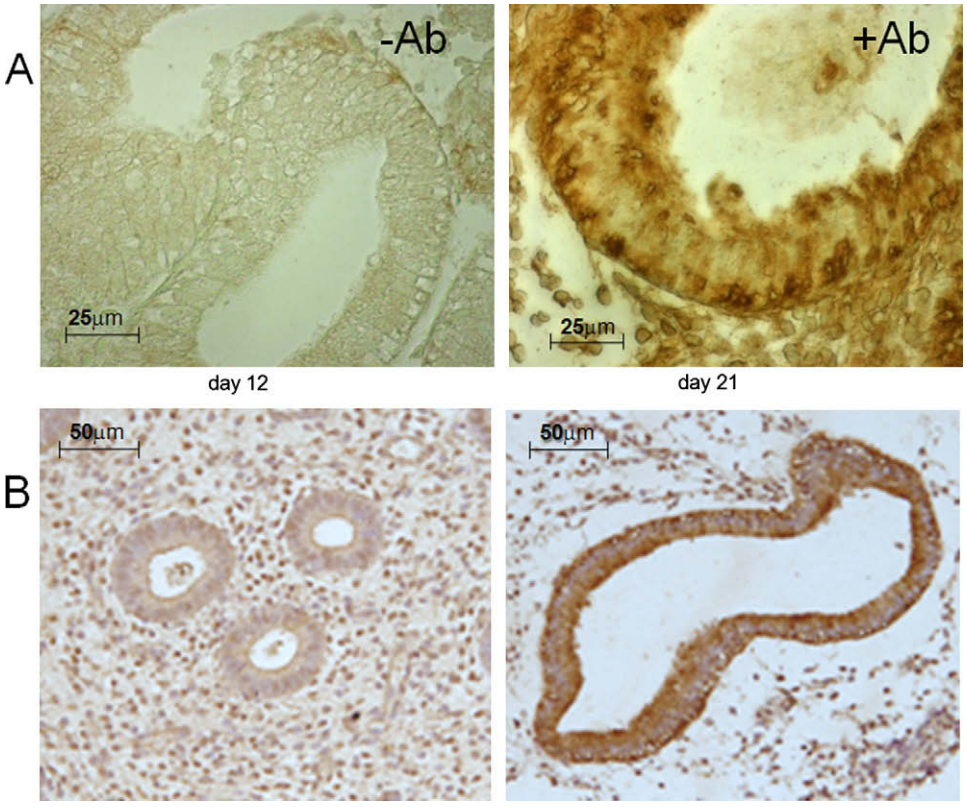
UPIb is a known member of a family of four glycoproteins, UPIa, UPIb, UPII, and UPIII, which construct the asymmetric unit membrane (AUM) in the mammalian bladder (23, 24). AUM plays an important role in normal bladder epithelial physiology. It forms plaques that cover the apical surface of the urothelium (28–30). These plaques strengthen and stabilize the urothelium, preventing rupture of the cells during bladder distention, and form a permeability barrier (31–33). UPIa and UPIb belong to the transpanin superfamily of proteins, which are characterized by four transmembrane domains and folding that creates one small intracellular and two, small and large, extracellular loops (24, 34). To form the AUM plaques, these two proteins undergo dimerization with UPII and UPIII to form complexes of UPIa/UPII and UPIb/UPIII (35).

The UPIb/UPIII complex is also expressed in nonmammalian systems such as the Xenopus egg. In this system, the UPIb/UPIII complex is involved in the sperm-egg membrane interaction and the subsequent egg activation via Src tyrosine kinase, which activates phospholipase c at fertilization (36, 37). It has been recently suggested that the signaling for egg activation is mediated via the ganglioside GM1 that interacts with UPIII in the complex (38). UPIII also has a tyrosine phosphorylation site (Tyr-249) in its carboxy-terminal cytoplasmic sequence (37) that is essential for sperm-induced egg activation (36). Since our analysis could not detect an increase in UPIII expression in human endometrium, it seems that these signaling pathways are not involved in the preparation of the endometrium for implantation.

Localization of UPIII in the membrane is absolutely dependent on its dimerization with UPIb (38), whereas UPIb is able to exit the endoplasmic reticulum (ER) and migrate to the plasma membrane as a monomer (39). This virtue of UPIb apparently allows its localization on the apical

FIGURE 3

Localization and temporal pattern of UPIb protein expression. Paraffin sections of endometrial samples were deparaffinized and stained immunohistochemically using antibodies to UPIb. (**A**) Endometrial glands of the secretory phase with antibodies (+Ab) show apical localization of UPIb on the cytoplasmic membranes as well as its localization on vacuolar membranes. Glands without antibodies (−Ab) do not stain. (**B**) Immunostaining of two endometrial samples taken from the same patient at the proliferative (day 12) and the secretory phase (day 21) of the cycle. These samples were also stained with hematoxylin.



Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

membrane of the glandular epithelium, even though this is the only member of the entire uroplakin family expressed in the endometrium. The localization of UPIb protein in the secretory vesicles of the glandular epithelium may suggest its involvement in the activity of the endometrial glands.

MUC1 has also been shown to be elevated in samples of biopsy-treated patients and is one of the predominant molecules secreted by the endometrial glands. Its level in the endometrium increases during the secretory phase of the cycle in response to high blood P levels (40). It plays a central role in the acquisition of endometrial receptivity and in regulation of implantation (41, 42). MUC1 continues to be secreted during early pregnancy and is taken by the syncytiotrophoblast via a phagocytotic mechanism (43).

The increase in UPIb mRNA levels on days 21–24 of the spontaneous menstrual cycle agrees with the idea that this protein is involved in the preparation of a receptive endometrium during the secretory phase. The additional increase in UPIb that was observed on days 20–21 of the following cycle further supports our assumption that biopsy treatment induces the expression of genes related to uterine receptivity. Such an increase was indeed observed in six out of 11 patients. The fact that this response is limited to 55% of the patients probably represents the heterogeneity of this group, which included patients whose infertility is not necessarily related to an endometrial defect.

Acknowledgments: The authors thank Dr. Shirley Horn-Saban and Dr. Ron Ophir for excellent assistance with DNA microarray analysis.

REFERENCES

1. Wang H, Dey SK. Road map to embryo implantation: clues from mouse models. Nat Rev Gen 2006;7:185–99.
2. Dunn CL, Kelly RW, Critchley HO. Decidualization of the human endometrial stromal cell: an enigmatic transformation. Reprod Biomed Online 2003;7:151–61.
3. Paria BC, Reese J, Das SK, Dey SK. Deciphering the cross-talk of implantation: advances and challenges. Science 2002;296:2185–8.

4. Dimitriadis E, Salmonsen LA, Robb L. Expression of interleukin-11 during the human menstrual cycle: coincidence with stromal cell decidualization and relationship to leukemia inhibitory factor and prolactin. Mol Hum Reprod 2000;6:907–14.
5. Cork BA, Li TC, Warren MA, Laird SM. Interleukin-11 (IL-11) in human endometrium: expression throughout the menstrual cycle and the effects of cytokines on endometrial IL-11 production in vitro. J Reprod Immunol 2001;50:3–17.
6. Dimitriadis E, Stoikos C, Baca M, Fairlie WD, McCoubrie JE, Salmonsen LA. Relaxin and prostaglandin E(2) regulate interleukin 11 during human endometrial stromal cell decidualization. J Clin Endocrinol Metab 2005;90:3458–65.
7. Akbas GE, Taylor HS. HOXC and HOXD gene expression in human endometrium: lack of redundancy with HOXA paralogs. Biol Reprod 2004;70:39–45.
8. Popovici RM, Kao LC, Giudice LC. Discovery of new inducible genes in in vitro decidualized human endometrial stromal cells using microarray technology. Endocrinology 2000;141:3510–3.
9. Carson DD, Lagow E, Thathiah A, Al-Shami R, Farach-Carson MC, Vernon M, et al. Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. Mol Hum Reprod 2002;8:871–9.
10. Kao LC, Tulac S, Lobo S, Imani B, Yang JP, Germeyer A, et al. Global gene profiling in human endometrium during the window of implantation. Endocrinology 2002;143:2119–38.
11. Dominguez F, Remohi J, Pellocer A, Simon C. Human endometrial receptivity: a genomic approach. Reprod Biomed Online 2003;6:332–8.
12. Giudice LC. Microarray expression profiling reveals candidate genes for human uterine receptivity. Am J Pharmacogenomics 2004;4:299–312.
13. Riesewijk A, Martin J, van Os R, Horcajadas JA, Polman J, Pellicer A, et al. Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. Mol Hum Reprod 2003;9:253–64.
14. Stewart CL, Kasper P, Brunet LJ, Bhatt H, Gadi I, Kontgen F, et al. Blastocyst implantation depends on maternal expression of leukemia inhibitory factor. Nature 1992;3(359):76–9.
15. Satokata I, Benson G, Maas R. Sexually dimorphic sterility phenotypes in Hoxa10 deficient mice. Nature 1995;37:460–3.
16. Benson GV, Lim H, Paria BC, Satokata I, Dey SK, Maas RL. Mechanisms of reduced fertility in Hoxa-10 mutant mice: uterine homeosis and loss of maternal Hoxa-10 expression. Development 1996;122:2687–96.
17. Nie G, Li Y, Wang M, Liu YX, Findlay JK, Salmonsen LA. Inhibiting uterine PC6 blocks embryo implantation: an obligatory role for a proprotein convertase in fertility. Biol Reprod 2005;72:1029–36.
18. Dimitriadis E, Stoikos C, Stafford-Bell M, Clark I, Pavia P, Kovacs, et al. Interleukin-11, IL-11 receptor alpha and leukemia inhibitory factor are dysregulated in endometrium of infertile women with endometriosis during the implantation window. J Reprod Immunol 2006;69:53–64.
19. Mikolajczyk M, Wirstlein P, Skrzypczak J. Leukemia inhibitory factor and interleukin 11 levels in uterine flushings of infertile patients with endometriosis. Hum Reprod 2006;21:3054–8.
20. Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I. Local injury of the endometrium doubles the incidence of successful pregnancies in patients undergoing in-vitro fertilization. Fertil Steril 2003;79:1317–22.
21. Raziel A, Schachter M, Strassburger D, Berno O, Ron-El R, Friedler S. Favorable influence of local injury to the endometrium in intracytoplasmic sperm injection patients with high-order implantation failure. Fertil Steril 2007;87:198–201.
22. Zhou L, Li R, Wang R, Huang HX, Zhong K. Local injury to the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates. Fertil Steril. Published online 6 August 2007. doi: 10.1016/j.fertnstert.2007.05.064. [E-pub ahead of print].
23. Wu XR, Manabe M, Yu J, Sun TT. Large scale purification and immunolocalization of bovine uroplakins I, II, and III. Molecular markers of urothelial differentiation. J Biol Chem 1990;265:19170–9.
24. Yu J, Lin JH, Wu XR, Sun TT. Uroplakins Ia and Ib, two major differentiation products of bladder epithelium, belong to a family of four transmembrane domain (4TM) proteins. J Cell Biol 1994;125:171–82.
25. Granot I, Dekel N, Bechor E, Segal I, Fieldust S, Barash A. Temporal analysis of connexin43, protein and gene expression, throughout the menstrual cycle in human endometrium. Fertil Steril 2000;73:381–6.
26. Horne AW, Lalani EN, Margara RA, Ryder TA, Mobberley MA, White JO. The expression pattern of MUC1 glycoforms and other biomarkers of endometrial receptivity in fertile and infertile women. Mol Reprod Dev 2005;72:216–29.
27. Dey SK. Fatty link to fertility. Nature 2005;435:34–5.
28. Hicks RM, Ketterer B. Hexagonal lattice of subunits in the thick luminal membrane of the rat urinary bladder. Nature 1969;224:1304–5.
29. Vergara JA, Longley W, Robertson JD. A hexagonal arrangement of subunits in membrane of mouse urinary bladder. J Mol Biol 1969;46:593–6.
30. Brisson A, Wade RH. Three-dimensional structure of luminal plasma membrane protein from urinary bladder. J Mol Biol 1983;166:21–36.
31. Negrete HO, Rivers RL, Goughs AH, Colombini M, Zeidel ML. Individual leaflets of a membrane bilayer can independently regulate permeability. J Biol Chem 1996;271:11627–30.
32. Lewis SA. Everything you wanted to know about the bladder epithelium but were afraid to ask. Am J Physiol 2000;278:F867–74.
33. Apodaca G. The uroepithelium: not just a passive barrier. Traffic 2004;5:117–28.
34. Helmer ME. Transpanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. Ann Rev Cell Dev Biol 2003;19:397–422.
35. Wu XR, Medina JJ, Sun TT. Selective interactions of UPIa and UPIb, Two members of the transmembrane 4 superfamily, with distinct single transmembrane-domained proteins in differentiated urothelial cells. J Biol Chem 1995;270:29752–9.
36. Mahbub Hasan AKM, Sato K, Sakakibara K, Ou Z, Iwasaki T, Ueda Y, et al. Uroplakin III, a novel Src subutrate in *Xenopus* egg rafts, is a target for sperm protease essential for fertilization. Dev Biol 2005;286:483–92.
37. Sakakibara K, Sato K, Yoshino K, Oshiro N, Hirahara S, Mahbub Hasan AKM, et al. Molecular identification and characterization of *Xenopus* egg uroplakin III, an egg raft-associated transmembrane protein that is tyrosine-phosphorylated upon fertilization. J Biol Chem 2005;280:15029–37.
38. Mahbub Hasan AKM, Ou Z, Sakakibara K, Hirahara S, Iwasaki T, Sato K, et al. Characterization of *Xenopus* egg membrane microdomains containing uroplakin Ib/III complex: roles of their molecular interactions for subcellular localization and signal transduction. Genes to Cells 2007;12:251–67.
39. Tu L, Sun TT, Kreibich G. Specific heterodimer formation is a prerequisite for uroplakins to exit from the endoplasmic reticulum. Mol Biol Cell 2002;13:4221–30.
40. Horne AW, Lalani EN, Margara RA, White JO. The effect of sex steroids hormones and interleuklin-1-beta on MUC1 expression in endometrial epithelial cell lines. Reproduction 2006;131:733–42.
41. Aplin JD. Adhesion molecules in implantation. Reproduction 1997;2:84–93.
42. Carson DD, Julian J, Lessey BA, Prakobphol A, Fisher SJ. MUC1 is a scaffold for selectin ligands in the human uterus. Front Biosci 2006;11:2903–8.
43. Burton GJ, Watson AL, Hempstock J, Skepper JN, Jauniax E. Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. J Clin Endocrinol Metab 2002;87:2954–9.

APPENDIX 1

TABLE 1

Genes up-regulated by endometrial biopsy.

Families (n) and description	Accession no.	Fold change	*P*
Cellular lipid metabolism (19):			
st3 beta-galactoside alpha-2,3-sialyltransferase 6 (SIAT10)	NM_006100	4.8/3.8	.0011
Hydroxysteroid (17-beta) dehydrogenase 2 (HSD17B2)	NM_002153	4.8	.0336
Aldehyde dehydrogenase 1 family, member A3 (ALDH1A3)	NM_000693	4.5	.0242
atp-binding cassette, sub-family g (white), member 1 (ABCG1)	NM_207627	4.1/3.4	.00585
Aldehyde dehydrogenase 3 family, member B2 (ALDH3B2)	NM_000695 NM_001031615	3.7	.00189
Sulfotransferase family 1e, estrogen-preferring, member 1 (SULT1E1)	NM_005420	3.2	.0269
Phospholipase c-like 1 (PLCL1)	NM_006226	2.9	.0266
acyl-coa synthetase long-chain family member 5 (ACSL5)	NM_016234 NM_203379 NM_203380	2.8	.0104
Hydroxysteroid (11-beta) dehydrogenase 2 (HSD11B2)	NM_000196	2.5	.0141
Apolipoprotein D (APOD)	NM_001647	2.5	.0368
Carnitine palmitoyltransferase 1a (liver) (CPT1A)	NM_001031847 NM_001876	2.4	.00080
Propionyl coenzyme A carboxylase, alpha polypeptide (PCCA)	NM_000282	2.4	.00401
udp-gal:betaglcnac beta 1,4-galactosyltransferase, polypeptide 4 (B4GALT4)	NM_003778 NM_212543	2.4	.0037
Protein kinase, amp-activated, gamma 2 non-catalytic subunit (PRKAG2)	NM_016203	2.4	.00174
24-dehydrocholesterol reductase (DHCR24)	NM_014762	2.4	.0202
3-hydroxy-3-methylglutaryl-coenzyme a reductase (HMGCR)	NM_000859	2.2	.0191
Apolipoprotein l, 2 (APOL2)	NM_030882 NM_145637	2.2/2	.00702
acyl-coa synthetase long-chain family member 4 (ACSL4)	NM_004458 NM_022977	2.2	.0106
Fatty-acid-coenzyme a ligase, long-chain 1 (ACSL1)	NM_001995	2.1	.0361
Carbohydrate metabolism (7):			
Glucosaminyl (N-acetyl) transferase 3, mucin type (GCNT3)	NM_004751	3.7	.0364
Pyruvate dehydrogenase kinase, isoenzyme 4 (PDK4)	NM_002612	2.8	.00381
Sorbitol dehydrogenase (SORD)	NM_003104	2.6/2.4	.0014
Genethonin 1 (GENX-3414)	NM_003943	2.3	.0151
Mannosidase alpha class 2B member 2 (KIAA0935)	NM_015274	2.2	.00024
Carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 4 (CHST4)	NM_005769	2.2	.00313

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

TABLE 1

Continued.

Families (n) and description	Accession no.	Fold change	*P*
Phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI) (PYGL)	NM_002863	2.1	.0272
Metallothionein superfamily (5):			
Metallothionein 1F (functional) (MT1F)	NM_005949	3.8/3.7	.0335
Metallothionein 1G (MT1G)	NM_005950	3.8	.0389
Metallothionein 1E (functional) (MT1E)	NM_175617	3.7/3.1	.0383
Metallothionein 1H (MT1H)	NM_005951	3.5/2.8	.0342
Metallothionein 1X (MT1X)	NM_005952	3.3	.0399
Amino acid metabolism/protein biosynthesis (9):			
Homogentisate 1,2-dioxygenase (homogentisate oxidase) (HGD)	XM_001123365	4.8/4.4/4.3	.0122
DnaJ (Hsp40) homolog, subfamily D, member 1 (DNAJD1)	NM_013238	3.6	.00532
Branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease) (BCKDHB)	NM_000056 NM_183050	3.3	.00019
Ribosomal protein S6 kinase, 90kDa, polypeptide 5 (RPS6KA5)	NM_004755 NM_182398	2.5	.0218
Aldehyde dehydrogenase 6 family, member A1 (ALDH6A1)	NM_005589	2.4/2.2	.00299
5-methyltetrahydrofolate-homocysteine methyltransferase (MTR)	NM_000254	2.3	.00161
4-aminobutyrate aminotransferase (ABAT)	NM_000663 NM_020686	2.2	.0254
Spermidine/spermine N1-acetyltransferase (SAT)	NM_002970	2.2	.0362
Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I (P4HA1)	NM_000917 NM_001017962	2.1	.00267
Transport (23):			
Solute carrier family 3, member 1 (SLC3A1)	NM_000341	5.1	.0311
Solute carrier family 39, member 14 (SLC39A14)	NM_015359	4.4	.0144
Solute carrier family 16 (monocarboxylic acid transporters), member 3 (SLC16A3)	NM_004207	3.5/3.0	.0266
Folate receptor 1 (adult) (FOLR1)	NM_000802 NM_016724 NM_016725 NM_016729 NM_016730 NM_016731	3.1	.00018
Solute carrier family 7 (cationic amino acid transporter, y+ system), member 4 (SLC7A4)	NM_004173	3.0	.00519
Elongation protein 3 homolog (S. cerevisiae) (ELP3)	NM_018091	2.7	.00654
Transmembrane 4 superfamily member 11 (plasmolipin) (TM4SF11)	NM_015993	2.7	.00434
Sodium channel, nonvoltage-gated 1 alpha (SCNN1A)	NM_001038	2.4	.00327
Solute carrier family 7 (cationic amino acid transporter, y+ system), member 1 (SLC7A1)	NM_003045	2.3	.0447

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

TABLE 1

Continued.

Families (n) and description	Accession no.	Fold change	*P*
Solute carrier family 4, sodium bicarbonate cotransporter, member 7 (SLC4A7)	NM_003615	2.3/772.1	.00539
SEC14-like 1 (S. cerevisiae) (SEC14L1)	NM_001039573 NM_003003	2.3/2.0	.00179
Hypothetical protein FLJ20551 (FLJ20551)	NM_017875	2.3	.00257
ATPase, Ca++ transporting, plasma membrane 2 (ATP2B2)	NM_001001331 NM_001683	2.3	.0033
Solute carrier family 16 (monocarboxylic acid transporters), member 1 (SLC16A1)	NM_003051	2.2	.013
Solute carrier family 43, member 1 (SLC43A1)	NM_003627	2.2	.00727
Vesicle-associated membrane protein 8 (endobrevin) (VAMP8)	NM_003761	2.2	.00069
ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1 (ATP6V1G1)	NM_004888	2.2	.0153
ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H (ATP6V1H)	NM_015941 NM_213619 NM_213620	2.2	5.17E-05
Nucleoporin 153kDa (NUP153)	NM_005124	2.2	.0164
Rab coupling protein (RCP)	NM_001002233 NM_001002814 NM_025151	2.2	0.045
Hypothetical protein FLJ20087	NM_017662	2.1	.00114
Solute carrier family 16 (monocarboxylic acid transporters), member 6 (SLC16A6)	NM_004694	2.0	.0304
ATPase, Na+/K+ transporting, alpha 1 polypeptide (ATP1A1)	NM_00701 NM_001001586	2.0	.00824
Antiapoptosis (8):			
KIAA0367	NM_015225	5.7/3.2	.00010
Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 9 (SERPINB9)	NM_004155	5.0	.00204
Phosphoprotein enriched in astrocytes 15 (PEA15)	NM_003768 NM_013287	3.0	.00126
Baculoviral IAP repeat-containing 3 (BIRC3)	NM_001165 NM_182962	2.8	.012
BCL2/adenovirus E1B 19kDa interacting protein 3-like (BNIP3L)	NM_004331	2.5	.00012
Forkhead box O1A (rhabdomyosarcoma) (FOXO1A)	NM_002015	2.5	.0418
Metallothionein-like 5, testis-specific (tesmin) (MTL5)	NM_001039656 NM_004923	2.2	.00132
Serum/glucocorticoid regulated kinase (SGK)	NM_005627	2.2	.041
Cell communication/signal transduction (17):			
Dual oxidase 1 (DUOX1)	NM_017434 NM_175940	10.3	.00529
Galanin (Gal)	NM_015973	5.2	.00541
Protein kinase, X-linked (PRKX)	NM_005044	4.1/3.9	.00541
Mitogen-activated protein kinase kinase 6 (MAP2K6)	NM_002758 NM_031988	3.9	.00352
Interferon gamma receptor 1 (IFNGR1)	NM_000416	3.8/2.4	.00415

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

TABLE 1

Continued.

Families (n) and description	Accession no.	Fold change	*P*
ras homolog gene family, member C like 1 (ARHCL1)	XM_350880	3.5	.00114
Cystatin SN (CST1)	NM_001898	3.4	.00146
Crystallin, alpha B (CRYAB)	NM_001885	3.1	.00259
Family with sequence similarity 13, member A1 (FAM13A1)	NM_014883 NM_001015045	2.9/2.8/2.2	.00955
NAD(P)H dehydrogenase, quinone 1 (NQO1)	NM_00903	2.7/2.6/2.6	.00131
c-mer proto-oncogene tyrosine kinase (MERTK)	NM_006343	2.5/2.2	.0415
Period homolog 2 (Drosophila) (PER2)	NM_003894 NM_022817	2.4	.022
Hydroxysteroid (11-beta) dehydrogenase 2 (HSD11B2)	NM_000196	2.5	.0141
Dehydrogenase/reductase (SDR family) member 7 (DHRS7)	NM_016029	2.3	.0283
Annexin A3 (ANXA3)	NM_005139	2.3	9.04E-05
Phosphodiesterase 9A (PDE9A)	NM_001001567 NM_001001585	2.1	.002
Protein tyrosine phosphatase, receptor type, O (PTPRO)	NM_002848	2.0	.0016
Extracellular matrix/proteolysis (6):			
Calpain 6 (CAPN6)	NM_014289	5.4/3.5	.00932
Elastin (supravalvular aortic stenosis, Williams-Beuren syndrome) (ELN)	NM_000501	3.5	.00812
Matrix metalloproteinase 26 (MMP26)	NM_021801	3.4	.00242
A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 8 (ADAMTS8)	NM_007037	2.6/6.9	.00724
Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5 (SERPINA5)	NM_000624	2.3	.000537
Calpain 3, (p94) (CAPN3)	NM_000070 NM_024344 NM_173087 NM_212464	2.2	.000636
Cell adhesion molecules (15):			
Cysteine-rich secretory protein 3 (CRISP3)	NM_006061	8.7	.0178
PDZ domain containing 3 (PDZK3)	NM_178140	4.1	.0103
CD36 antigen (collagen type I receptor, thrombospondin receptor) (CD36)	NM_001001547	3.7/3.5	.0491
Epithelial V-like antigen 1 (EVA1)	NM_144765	3.7/3.1	.00628
Catenin (cadherin-associated protein), alpha 2 (CTNNA2)	NM_004389	3.7	.00966
Integrin, beta 8 (ITGB8)	NM_002214	2.6	.00352
Myosin X (MYO10)	NM_012334	2.2	.000348
Junction plakoglobin (JUP)	NM_002230 NM_021991	2.5	.0102
Discs, large homolog 5 (Drosophila) (DLG5)	NM_004747	2.3	.0105
Plakophilin 2 (PKP2)	NM_001005242 NM_004572	2.2	.00251

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

TABLE 1

Continued.

Families (n) and description	Accession no.	Fold change	*P*
Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) (SPOCK)	NM_004598	2.1	.00938
CD9 antigen (p24) (CD9)	NM_001769	2.1	.00667
Transmembrane protein 8 (five membrane-spanning domains) (TMEM8)	NM_021259	2.1	.000928
Desmocollin 2 (DSC2)	NM_004949 NM_024422	2.1	.0252
Cell organization/cytoskeleton (12):			
Keratin 8 (KRT8)	NM_002273	3.3	.00131
Periplakin (PPL)	NM_002705	2.8	.00368
Nebulette (NEBL)	NM_006393 NM_213569	2.7/2.6	.00226
Nephroblastoma overexpressed gene (NOV)	NM_002514	2.6	.0146
Gelsolin (amyloidosis, Finnish type) (GSN)	NM_000177 NM_198252	2.5	.0159
Keratin 23 (histone deacetylase inducible) (KRT23)	NM_015515	2.5	.0498
Keratin 18 (KRT18)	NM_000224 NM_199187	2.5	.00287
Microtubule-associated protein 7 (MAP7)	NM_003980	2.4	.00108
RAN binding protein 17 (RANBP17)	NM_022897	2.4	.0187
Keratin 19 (KRT19)	NM_002276	2.3	.0102
Erythrocyte membrane protein band 4.1 like 4B (EPB41L4B)	NM_01842 NM_019114	2.3	1.26E-05
Ankyrin 3, node of Ranvier (ankyrin G) (ANK3)	NM_001149 NM_020987	2.2	.0329
Immune/wound response (5):			
Tissue factor pathway inhibitor 2 (TFPI2)	NM_006528	5.0	.0164
Secretoglobin, family 1D, member 2 (SCGB1D2)	NM_006551	2.9	.0198
Interleukin 20 receptor, alpha (IL20RA)	NM_014432	2.9	.00525
Toll-like receptor 5 (TLR5)	NM_003268	2.5	.00027
B-cell scaffold protein with ankyrin repeats 1 (BANK1)	NM_017935	2.2	.00046
Transcription regulation/DNA metabolism (21):			
Tumor protein D52-like 1 (TPD52L1)	NM_003287	4.7	.00358
Nnuclear factor I/B (NFIB)	NM_005596	3.3	.00132
Interferon stimulated gene 20kDa (ISG20)	NM_002201	3.3	.0154
Zinc finger CCCH type, antiviral 1 (ZC3HAV1)	NM_020119 NM_024625	3.2	.026
Nuclear receptor subfamily 4, group A, member 2 (NR4A2)	NM_006186 NM_173171	3.2	.00819
Uridine monophosphate kinase (UMPK)	NM_012474	3.1	.00015
Paired box gene 2 (PAX2)	NM_000278 NM_003987	2.9	.00294
Ectonucleoside triphosphate diphosphohydrolase 3 (ENTPD3)	NM_001248	2.9	.00647
Cartilage intermediate layer protein, nucleotide pyrophosphohydrolase (CILP)	NM_003613	2.9	.0107

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

TABLE 1

Continued.

Families (n) and description	Accession no.	Fold change	*P*
Guanosine monophosphate reductase (GMPR)	NM_006877	2.8	.00217
Pirin (PIR)	NM_001018109 NM_003662	2.7	.0022
Transcription factor CP2-like 1 (TFCP2L1)	NM_014553	2.6	.00052
cAMP responsive element binding protein 3-like 1 (CREB3L1)	NM_052854	2.6	.0354
v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian) (MAFF)	AA803125	2.6	.0451
Rho-related BTB domain containing 3 (RHOBTB3)	NM_014899	2.4	.00415
Thiamin pyrophosphokinase 1 (TPK1)	NM_022445	2.4	.00517
BTAF1 RNA polymerase II, B-TFIID transcription factor-associated, 170kDa (Mot1 homolog, S. cerevisiae) (BTAF1)	NM_003972	2.3	.000754
Delta sleep inducing peptide, immunoreactor (DSIPI)	NM_001015881 NM_004089 NM_198057	2.3	.0205
Zinc finger, BED domain containing 2 (ZBED2)	NM_024508	2.2	.000127
PHD finger protein 8 (KIAA1111)	NM_015107	2.1	.00323
Adenosine kinase (ADK)	NM_001123 NM_006721	2.1	2.41E-05
PG biosynthesis (2):			
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) (PTGS1)	NM_000962	3.5	.00841
Phospholipase A2, group IVA (cytosolic, calcium-dependent) (PLA2G4A)	NM_024420	2.5	.000577
Cell cycle (5):			
Acid phosphatase, prostate (ACPP)	NM_001099	2.8	.0207
Transforming, acidic coiled-coil containing protein 2 (TACC2)	NM_206862 NM_006997	2.8	.00946
CDC14 cell division cycle 14 homolog B (S. cerevisiae) (CDC14B)	NM_003671 NM_033331	2.5	.00145
Lethal giant larvae homolog 2 (Drosophila) (LLGL2)	NM_001015002 NM_001031803 NM_004524	2.2	.000989
nth endonuclease III-like 1 (E. coli) (NTHL1)	NM_000548 NM_021055 NM_021056	2.1	.00156
Cell surface/membrane (4):			
Uroplakin 1B (UPK1B)	NM_006952	7.4/6.7	.00114
Adipose differentiation-related protein (ADFP)	NM_001122	2.3	.00166
Lysosomal-associated membrane protein 2 (LAMP2)	NM_002294 NM_013995	2.1	.00235
Mucin 1, transmembrane (MUC1)	NM_001018016 NM_001018017 NM_001018021 NM_002456	2.1	.029
Cellular metabolism/electron transport (3):			
Cytochrome P450, family 26, subfamily A, polypeptide 1 (CYP26A1)	NM_000783 NM_057157	9.0	.0109

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

TABLE 1

Continued.

Families (n) and description	Accession no.	Fold change	*P*
Flavin containing monooxygenase 5 (FMO5)	NM_001461	2.8	.00093
Cytochrome b-5 (CYB5)		2.4	.0164
Autophagy (2):			
WD repeat domain, phosphoinositide interacting 1 (FLJ10055)	NM_017983	2.6	.0302
Microtubule-associated protein 1 light chain 3 beta (MAP1LC3B)	NM_022818	2.2	.0194
Other cellular functions (20):			
Secretoglobin, family 2A, member 2 (SCGB2A2)	NM_002411	5.5	.0257
Leucine rich repeat containing 1 (LRRC1)	NM_018214	4.0	.00772
Hypothetical protein FLJ21511	NM_025168	3.8	.00311
Cysteine-rich secretory protein 2 (CRISP2)	NM_003296	3.4	.00549
Carbonyl reductase 3 (CBR3)	NM_001236	3.1	.00945
Chromosome 21 open reading frame 68	NM_024944	2.9	.00458
Proline rich 1 (PROL1)	NM_021225	2.7	.0118
FK506 binding protein 5 (FKBP5)	NM_004117	2.5	.00473
Xanthine dehydrogenase (XDH)	NM_000379	2.4	.0312
KIAA0826 protein	XM_093839	2.4	.00017
Creatine kinase, brain (CKB)	NM_001823	2.3	.0166
TED protein (TED)	NM_015686	2.3	.00087
Hypothetical protein FLJ20366	NM_017786	2.3	.00128
Sulfide quinone reductase-like (yeast) (SQRDL)	NM_021199	2.2	.00778
Ras-related GTP binding D (RRAGD)	NM_021244	2.2	.00090
Chromosome 11 open reading frame 8 (C11orf8)	NM_001584	2.2	.0242
Troponin C, slow (TNNC1)	NM_003280	2.1	.00495
CASK-interacting protein CIP98 (CIP98)	NM_015404	2.1	.0129
DNA-damage-inducible transcript 4 (DDIT4)	NM_019058	2.1	.0397
Hypothetical protein dJ465N24.2.1	NM_020317 NM_207035	2.0	.0434

Note: Total RNA was extracted from endometrial samples of four biopsy-treated patients who conceived in the subsequent IVF treatment and four control patients. Each RNA extract was separately hybridized to a chip. The fold change values were calculated as the ratio between the mean values of the four treated and control samples. Significant changes with a fold change more than 2 are presented. In cases in which genes were present twice on the array, both values of fold of induction are presented.

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

TABLE 2

Genes down-regulated by endometrial biopsy.

Families (no.) and description	Accession no.	Fold change	*P*
Cellular metabolism (2):			
Aldehyde dehydrogenase 1 family, member A1 (ALDH1A1)	NM_000689	2.1	.0284
Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1)	NM_004181	2.1	.00024
Cell growth inhibition (1):			
WAP four-disulfide core domain 1 (WFDC1)	NM_021197	2.5	.012
Cell communication/signal transduction (7):			
Thyrotropin-releasing hormone (TRH)	NM_007117	3.9	.0424
Secreted frizzled-related protein 1 (SFRP1)	NM_003012	2.9	.0446
Lymphoid enhancer-binding factor 1 (LEF1)	NM_016269	2.4	.0441
Phosphoinositide-3-kinase, regulatory subunit, polypeptide 3 (p55, gamma) (PIK3R3)	NM_003629	2.3	.0355
Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1) (CXCL12)	NM_000609	2.2	.0031
Gap junction protein, alpha 4, 37kDa (connexin 37) (GJA4)	NM_002060	2.1	.0383
G protein-coupled receptor 30 (GPR30)	NM_001031682 NM_001505	2.0	.0495
Extracellular matrix/proteolysis (4):			
Matrix metalloproteinase 11 (stromelysin 3) (MMP11)	NM_005940	5.2	.0309
DKFZP586H2123 protein	NM_001001991 NM_015430	2.8	.0459
Fibrillin 2 (congenital contractural arachnodactyly) (FBN2)	NM_001999	2.8	.05
Carboxypeptidase Z (CPZ)	NM_001014447 NM_001014448 NM_003652	2.2	.00922
Cell adhesion molecules (2):			
Transforming growth factor, beta-induced, 68kDa (TGFBI)	NM_000358	3.1	.0366
Periostin, osteoblast specific factor (POSTN)	NM_006475	3.1	.0229
Cell organization/cytoskeleton (2):			
Kinesin family member 20A (KIF20A)	NM_005733	2.3	.0464
Palladin (KIAA0992)	NM_016081	2.0	.0158
Immune/wound response (3):			
Coagulation factor XIII, A1 polypeptide (F13A1)	NM_000129	3.0	.0429
Natural killer cell transcript 4 (NK4)	NM_00101263–6 NM_001012718 NM_004221	2.4	.0349
Thy-1 cell surface antigen (THY1)	NM_006288	2.1	.0336
Transcription regulation/DNA metabolism (2):			
Thymidylate synthetase (TYMS)	NM_001071	3.5	.0296
Ribonucleotide reductase M2 polypeptide (RRM2)	NM_001034	3.2	.0273
Transport (2):			
Hemoglobin, alpha 1 (HBA1)	NM_000558	2.4	.0118
Hemoglobin, alpha 2 (HBA2)	NM_000517	2.4	.013
Cell cycle (9):			
Nucleolar and spindle associated protein 1 (NUSAP1)	NM_016359 NM_018454	2.8	.0484
Patched homolog (Drosophila) (PTCH)	NM_000264	2.7	.0165

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.

TABLE 2

Continued.

Families (no.) and description	Accession no.	Fold change	*P*
Discs, large homolog 7 (Drosophila) (DLG7)	NM_014750	2.5	.0433
Ubiquitin-conjugating enzyme E2C (UBE2C)	NM_007019 NM_181799 -83	2.4	.0252
TPX2, microtubule-associated protein homolog (Xenopus laevis) (TPX2)	NM_012112	2.3	.0293
Centromere protein F, 350/400ka (mitosin) (CENPF)	NM_016343	2.3	.0385
Baculoviral IAP repeat-containing 5 (survivin) (BIRC5)	NM_001012270 NM_001012271 NM_001168	2.2	.013
Cyclin D2 (CCND2)	NM_001759	2.1	.00094
ZW10 interactor (ZWINT)	NM_001005413 NM_007057 NM_032997	2.0	.0435
Other cellular functions (5):			
KIAA0101 gene product (KIAA0101)	NM_001029989 NM_014736	2.5	.05
Hypothetical protein FLJ10781 (FLJ10781)	NM_018215	2.3	.0468
Four jointed box 1 (Drosophila) (FJX1)	NM_014344	2.2	.0193
Mesoderm specific transcript homolog (mouse) (MEST)	NM_002402 NM_177524	2.1	.0444
Chromosome 13 open reading frame 18 (C13orf18)	NM_025113	2.0	.0349

Note: Total RNA was extracted from endometrial samples of four biopsy-treated patients who conceived in the subsequent IVF treatment and four control patients. Each RNA extract was separately hybridized to a chip. The fold change values were calculated as the ratio between the mean values of the four treated and control samples.

Kalma. Expression of UPIb in human endometrium. Fertil Steril 2009.